Name of Mouse model or mutation:
APP-MABH-EM7-B6

Description:
Series of point mutations introduced using CRISPR/Cas9 gene editing.

Type of mutation:
SNP: SNPs: G601R, F606Y, R609H

Delivery method:
Cytoplasmic injection into 1-cell stage embryo

Genetic Background:
C57BL/6J

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
GCAGAATTCGGACATGATTC	AGG
TGCCTACCAGTTTTTGATGG	CGG
CATCAAGACGGAAGAGATCT	CGG

lssDNA donor sequence (5'-3'):
AGTTTTTGCCTCCTTGTGGCTGGCGGTCACACTAACGGATGGCCCTGCATACTTTGTGTTTGACGCAG
GTTCTGGGCTGACAAACATCAAGACGGAAGAGATCTC**c**GAAGTGAAGATGGATGCAGAATTC**A**GAC
ATGATTCAGGAT**A**TGAAGT**t**C**A**CCATCAAAAACTGGTAGGCAAAAATAAACTGCCTCTCCCCGAGAT
TGCGTCTGGCCAGATGAAATACGTGGCACCTCGTGGCTTGTCCTGTGTC

* Bold and underlined in red indicates coding changes, bold and in red indicates silent changes introduced to prevent re-processing of the engineered allele by CRISPR/Cas9.

Microinjection mixes:
Microinjection buffer (MIB; 10 mM Tris–HCl, 0.1 mM EDTA, 100 mM NaCl, pH7.5) was prepared and filtered through a 2 µm filter and autoclaved. Cas9 mRNA, sgRNAs and lssDNA donors were diluted and mixed in MIB to the working concentrations of 50 ng/µl, 6.5 ng/µl each and 100 ng/µl, respectively. Injected embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F_0 progeny.

Sequence details

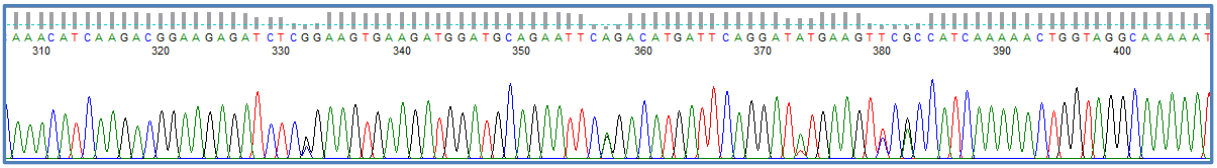
WT

AACCTGGCATCTTAACCAGCATTGATTTTTCCCCCCTTTCCCTCCCTCCCCTTTTAATTATGGCATGTGT
TTGGCCACACAGGCATTACATATTCAGCGTTCTTCAGTCTTTTTGTTTGCTAGGTGGTGGTTAATGGT
TTAAATCTAGGGTGTCATTTTTCTTGAGAAAAATCCCTAAATCCGTTTTTCCTAAAAGCTCTGGCCAA
GAGAGAACTTTAAGGCAGTTTTTGCCTCCTTGTGGCTGGCGGTCACACTAACGGATGGCCCTGCATA
CTTTGTGTTTGACGCAGGTTCTGGGCTGACAAACATCAAGACGGAAGAGATCTCGGAAGTGAAGAT
GGATGCAGAATTCGGACATGATTCAGGATTTGAAGTCCGCCATCAAAAACTGGTAGGCAAAAATAA
ACTGCCTCTCCCCGAGATTGCGTCTGGCCAGATGAAATACGTGGCACCTCGTGGCTTGTCCTGTGTC
AAAACTGAGAAGGACTACTGGGAATAAAACCAAAATGCCTGCCTAGATCTTCACAAAGATAGGAAG
GAGAGGAAGTGGGGCTCTGTTGATAGTTCTTGCTGAGCAGAAGCCGCTGAGCCCAGGCGGAACAT
ACAAGTGTAATTCAGTCCAACGTTGCAGCTACGTGAGCTGACTTCCTAGGAAAATGGTTTTTTCGGG
TTAAACACATCTAATCCCAATATCCAAACTGGAAGGCAGGATACCACAAACAAGAAATTCAAGTCCA
TGAGGCTCTGATATTACTGTGTGGGGGAAGCATAAGTGAATTTCATTTAGAGGTGGTCCCAGTTTCC
AAGATGGTCTCATTATGTAAATACCAAAAAAAAAAAAAAGTTAGAAATCAAGATTTTCGAAACCCTT
TTGGTCATGACCATTCTCGGTCCACGA

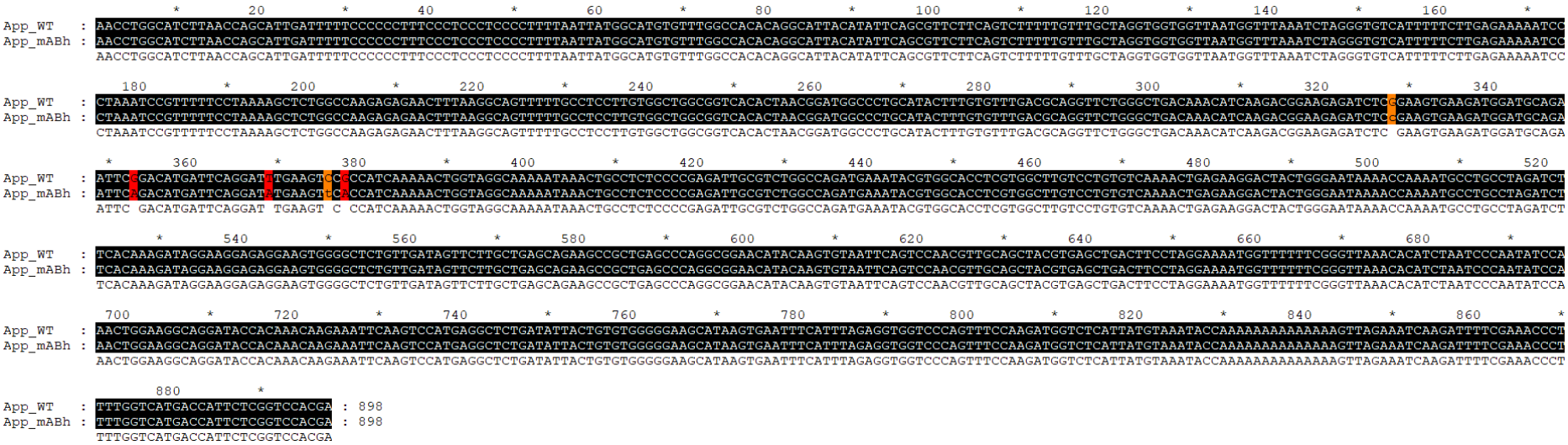
APP-MABH-EM7-B6

AACCTGGCATCTTAACCAGCATTGATTTTTCCCCCCTTTCCCTCCCTCCCCTTTTAATTATGGCATGTGT
TTGGCCACACAGGCATTACATATTCAGCGTTCTTCAGTCTTTTTGTTTGCTAGGTGGTGGTTAATGGT
TTAAATCTAGGGTGTCATTTTTCTTGAGAAAAATCCCTAAATCCGTTTTTCCTAAAAGCTCTGGCCAA
GAGAGAACTTTAAGGCAGTTTTTGCCTCCTTGTGGCTGGCGGTCACACTAACGGATGGCCCTGCATA
CTTTGTGTTTGACGCAGGTTCTGGGCTGACAAACATCAAGACGGAAGAGATCTC**c**GAAGTGAAGAT
GGATGCAGAATTC**A**GACATGATTCAGGAT**A**TGAAGT**t**C**A**CCATCAAAAACTGGTAGGCAAAAATAA
ACTGCCTCTCCCCGAGATTGCGTCTGGCCAGATGAAATACGTGGCACCTCGTGGCTTGTCCTGTGTC
AAAACTGAGAAGGACTACTGGGAATAAAACCAAAATGCCTGCCTAGATCTTCACAAAGATAGGAAG
GAGAGGAAGTGGGGCTCTGTTGATAGTTCTTGCTGAGCAGAAGCCGCTGAGCCCAGGCGGAACAT
ACAAGTGTAATTCAGTCCAACGTTGCAGCTACGTGAGCTGACTTCCTAGGAAAATGGTTTTTTCGGG
TTAAACACATCTAATCCCAATATCCAAACTGGAAGGCAGGATACCACAAACAAGAAATTCAAGTCCA
TGAGGCTCTGATATTACTGTGTGGGGGAAGCATAAGTGAATTTCATTTAGAGGTGGTCCCAGTTTCC
AAGATGGTCTCATTATGTAAATACCAAAAAAAAAAAAAAGTTAGAAATCAAGATTTTCGAAACCCTT
TTGGTCATGACCATTCTCGGTCCACGA

APP-MABH-EM7-B6 Heterozygous F1 animal sequence trace:

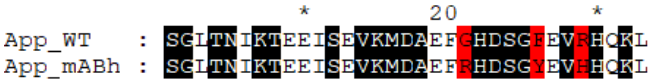


Nucleotide Alignment:



Orange changes highlight silent changes, Red highlights the coding changes G601R, F606Y, R609H.

Predicted Protein Alignment:



Red highlights the coding changes G601R, F606Y, R609H.

QC strategy employed at Harwell to check the edited allele:

Genomic DNA was extracted from ear clip biopsies and amplified in a PCR reaction using the following conditions/primer sequences:

Geno_ _(5'-3')	AACCTGGCATCTTAACCAGCA
Geno_ _ (5'-3')	GAGGAGGGGTTGGAATGGAC
Taq Polymerase used	ThermoFisher SuperFi II kit
Annealing Temperature (°C)	60
Elongation time (min)	0.75
WT product size (bp)	1028
Mutant product size (bp)	1028
Notes	Sequence with primers Geno_App_F2 (TTGGCCACACAGGCATTACA) and Geno_App_R3_Seq (TAGGAAGTCAGCTCACGTAG)

All amplicons were sent for Sanger sequencing to check for integration of the donor oligo sequence at the target site. F1 sequences should be heterozygous unless on sex chromosome.

Off-target site with ≤2 mismatches in seed sequence for guides used were checked with the following primers:

The following off-target sites with two or fewer mismatches in the seed sequence of the protospacer on the same chromosome as the target were checked by PCR and Sanger sequencing, with no evidence of off-target activity detected.

Off-target site	Sequence	Type	Primers used (5'-3')
16:22558354-22558376	**A**CAGAATTCGGACATG**T**TT**G** GGG	Intronic	Geno_App_OT1F1 primer: GGCAGCATCCCTGAGAACAT Geno_App_OT1R1 primer: TACCCCACCATCCCCTACTG
16:8472015-8472037	**A**CA**A**AA**A**TC**A**GACATGATTC AGG	Intronic	Geno_App_OT2F1 primer: CTGATTCCCAGGCGAAACCC Geno_App_OT2R1 primer: ACGTGGTTGGTGTGCTTACG Sequenced with Geno_App_OT2seqF (CTATCCTCTTATTCATGGTCAGTG) & Geno_App_OT2seqR (GCGCCAACCCAGAAAGTTCTTCTC)

16:5199485-5199507	CACCCTGAGGGAAGAGATCT CGG	Intronic	Geno_App_OT3F2 primer: CTCAGGTCCTTCGCTTGCGAGGC Geno_App_O31R1 primer: ACCCCTACCTTCCAGTCGTT Reverse sequenced with Geno_App_OT3seqR (TGCGTCAACAAGATGTCGTCAATG)
16:11025830-11025852	CGTCCAGACTGAAGAGAGCT TGG	Intronic	Geno_App_OT4F1 primer: CAATAGCAAACCAGGATGGAG Geno_App_OT4R1 primer: ACCCCTACCTTCCAGTCGTT Sequenced with Geno_App_OT4seqF (CCTTACACTTCCACATTCATCAG) & Geno_App_OT4seqR (AGTCTCTGGAAATTGTCCCTTG)

Additional integrations of the donor sequence

Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor oligos were inserted once on target into the genome. The following Taqman assay was used to copy count the donor sequence compared against a VIC-labelled reference assay for Dot1l:

Assay name	App-mABh-UPL34
Forward Primer (5'-3')	CATCAAAAACTGGTAGGCAAAA
Reverse Primer (5'-3')	GTGCCACGTATTTCATCTGG
Probe (5'-3')	UPL34
Label	FAM-BHQ1

This ddPCR assay is universal to App and both WT and mABh alleles are recognised by this assay. WT controls are expected to call at 2 copies and a single integration for a correct mutation is expected to call at 2 copies for F1 (HET) animals.

Assay name	App-mABh-MUT1
Forward Primer (5'-3')	GCCCTGCATACTTTGTGTTTGAC
Reverse Primer (5'-3')	CCTACCAGTTTTTGATGGTGAACTTCAT
Probe (5'-3')	ATCCTGAATCATGTCTGAATTCT
Label	FAM-BHQ1

This ddPCR assay is specific to App-mABh and only mutant alleles are recognised by this assay. WT controls are expected to call at 0 copies and a single integration for a correct mutation is expected to call at 1 copy for F1 (HET) animals.

Reference Assay Name	Dot1l
Forward primer (5'-3')	GCCCCAGCACGACCATT
Reverse primer (5'-3')	TAGTTGGCATCCTTATGCTTCATC
Probe (5'-3')	CCCAACAGGCCTGGATTCTCAATGC
Label	VIC

VIC-labelled reference assay for Dot1l gene.

No evidence of random donor insertions was observed using these assays in the animals used to establish the colony.

APP-MABH-EM1-B6N
APP-MABH-EM2-B6N
APP-MABH-EM3-B6N

Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; G601R, F606Y and R609H in exon ENSMUSE00000131684 of *App*. The stock was generated at MRC Harwell via microinjection of CRISPR/Cas9 reagents into 1-cell stage embryos.

qPCR Copy Counting Genotyping Strategy

The genotyping strategy presented here has been optimized for reagents and conditions used by the Genotyping Core at MRC Harwell. To genotype animals, we recommend researchers validate the assay independently. PCR cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Samples are genotyped using qPCR copy counting with both a wild type and a mutant assay against a known reference assay (*Dot1l* on chromosome 10; 2 copies present). Samples for this line are genotyped using the following primers and probe:

- Wild type (WT) assay with probe and reverse primer binding to the WT bases mutated in the mutant allele.
- Mutant assay with probe and reverse primer binding to the G601R, F606Y and R609H point mutations.

For autosomal genes that have been targeted, the following results would be expected:

Genotype of the Modified allele	WT Assay	Mutant Assay
Wildtype	2	0
Heterozygous	1	1
Homozygous mutant	0	2

APP-MABH-EM1-B6N
APP-MABH-EM2-B6N
APP-MABH-EM3-B6N

App-mABh-WT1 assay (FAM labelled)

CACACTAACGGATGGCCCTGCATACTTTGTGTTTGACGCAGGTTC**TGGGCTGACAAACATCAAGAC**GGAAGA
GA**TCTCgGAAGTGAAGATGGATGCAGAA**TTCgGACAT**GATTCAGGATtTGAAGTcCgCC**ATCAAAAACTGGTA
GGCAAAAATAAACTG

Lower case letters denote bases changed in the mutant allele.
Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
App-mABh-WT_F	n/a	**TGGGCTGACAAACATCAAGAC**	n/a	Wild type Forward
App-mABh-WT_PROBE	FAM	**TCTCGGAAGTGAAGATGGATGCAGAA**	ZEN/IBFQ	Wild type Probe
App-mABh-WT_R	n/a	**GGCGGACTTCAAATCCTGAATC**	n/a	Wild type Reverse

App-mABh-MUT1 assay (FAM labelled)

CACACTAACGGATG**GCCCTGCATACTTTGTGTTTGAC**GCAGGTTCTGGGCTGACAAACATCAAGACGGAAGA
GATCTCcGAAGTGAAGATGGATGC**AGAATTCaGACATGATTCAGGAT**aTGAAGTtCaCCATCAAAAACTGGT
AGGCAAAAATAAACTG

Lower case letters denote bases changed in the mutant allele.
Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
App-mABh-MUT_F	n/a	**GCCCTGCATACTTTGTGTTTGAC**	n/a	Mutant Forward
App-mABh-MUT_PROBE	FAM	**ATCCTGAATCATGTCTGAATTCT**	BHQ	Mutant Probe
App-mABh-MUT_R	n/a	**CCTACCAGTTTTTGATGGTGAACTTCAT**	n/a	Mutant Reverse

APP-MABH-EM1-B6N
APP-MABH-EM2-B6N
APP-MABH-EM3-B6N

Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACTA**CTAACA GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT CACCCTATGA

Oligo Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dot1l_Forward	n/a	**GCCCCAGCACGACCATT**	n/a	WT Forward
Dot1l_Probe	VIC	**CCAGCTCTCAAGTCG**	BHQ	WT Probe
Dot1l_Reverse	n/a	**TAGTTGGCATCCTTATGCTTCATC**	n/a	WT Reverse

Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

DNA extraction method

DNA is extracted from ear clips using Applied Biosystems Taqman Sample-to-SNP Kit and qPCR run using 1:10 dilution from the crude preparation.

qPCR master mix 1X

Applied Biosystems GTX Taqman master mix	5 µl
Dot1l_Forward (20 µM)	0.225 µl
Dot1l_Reverse (20 µM)	0.225 µl
Dot1l_Probe (5 µM)	0.2 µl
FAM Assay (probe 5 µM & primers 15 µM each)	0.3 µl
ddH20	1.55 µl
DNA (1:10 dilution of ABI Sample-to-SNP prep)	2.5 µl

Each sample is ran in technical duplicate. Seven WT and/or mutant controls are also included in duplicate along with non-template controls.

qPCR cycling conditions

qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

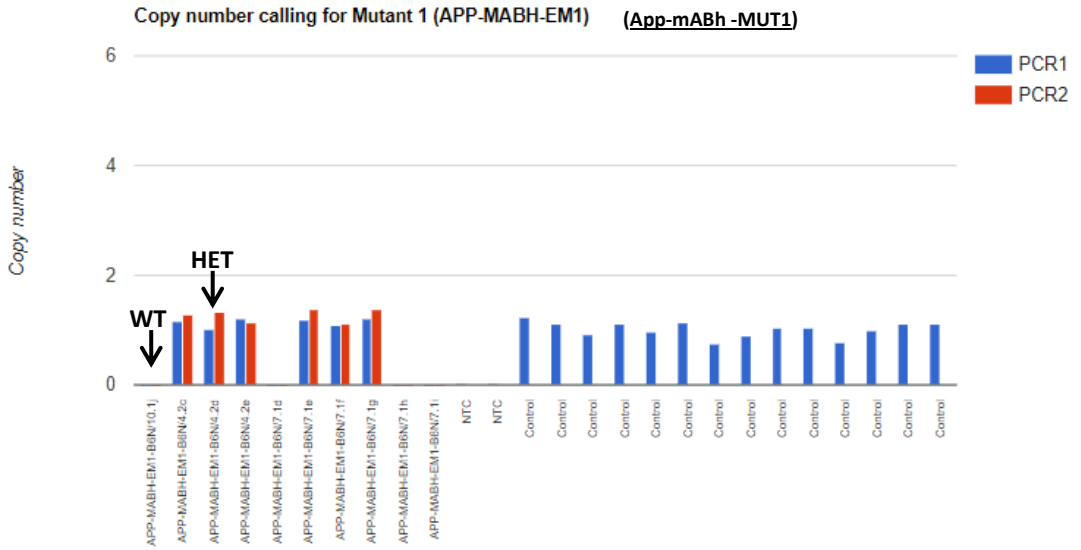
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

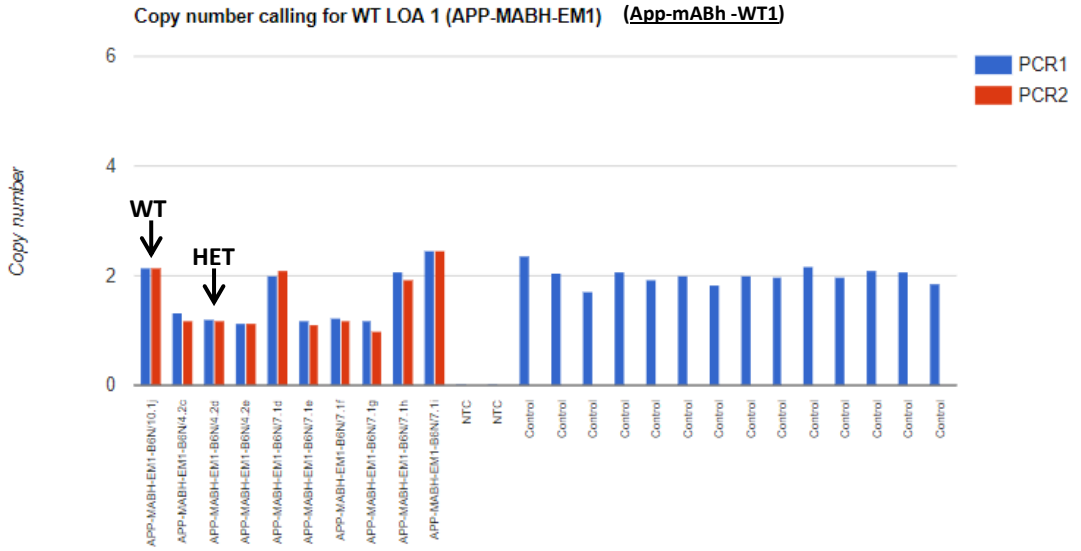
APP-MABH-EM1-B6N
APP-MABH-EM2-B6N
APP-MABH-EM3-B6N

Analysis

The results are analysed using CopyCaller software v2.0 from Applied Biosystems or in-house software that is based on CopyCaller v2.0.

App-mABh-WT1 and App-mABh -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 272666 results)





APP-MABH-EM1-B6N
APP-MABH-EM2-B6N
APP-MABH-EM3-B6N

Version No.	1
Date:	25.01.2020
Created/Updated by:	AC
Approved by:	Rumana Zaman